UNIVERSITI PUTRA MALAYSIA

BIOASSAY-GUIDED ISOLATION AND IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM GARCINIA PENANGIANA LEAVES

MOHD LIP BIN JABIT.

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By

MOHD LIP BIN JABIT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

December 2005

DEDICATION

My family

&

Friends

Many thanks for your support and inspiration

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Nordin Hj Lajis, PhD

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Preliminary screening was done on 18 extracts of different parts of *Garcinia sp.* These extracts were tested on cytotoxic assay by using MTT Tetrazolium method on MCF-7 cells (hormone dependent breast cancer cells), DU145 (prostate cancer cells), H460 (non-small lung cancer) and HL60 (Leukemic cancer cells). Extracts of *G. penangiana* leaves, *Garcinia urophylla* leaves, *G. maingayi* leaves, *G.maingayi* stems and *G. opaca* fruits were found to have potent cytotoxic activity on MCF-7 cells and their IC_{50} values are 5, 3, 6, 10 and 8 μg/mL, respectively. Furthermore, the extract of *G. penangiana* leaves also showed potent cytotoxic activity towards H460 cells (IC_{50} value of 8 μg/mL).

Bioassay guided isolation and purification led to the isolation of five xanthones and triterpene compounds from *Garcinia penangiana* leaves extracts. The triterpene and sterol isolated were charaterized as friedelin **(23)** and stigmasterol **(24)**, respectively. The two isolated xanthones from the hexane fraction were characterized as 1,3,5,8-tetrahydroxy-4-(1,1-dimethyl allyl)xanthone **(25)** and cudratricusxanthone H **(26)**. The

two xanthones isolated from the dichloromethane extract were characterised as 1,3,5,6-tetrahydroxy-2-(1,1-dimethylallyl)-4-(3-methyl-2-butenyl)xanthone or macluraxanthone C **(27)** and the new penangianaxanthone **(25)**. Compound designated as **29** was also found as a mixture of **27** in dichloromethane fraction. The biosynthesis of **25**, **26** and **28** was suggested in the discussion. These compounds were tested for cytotoxic assay by using MTT Tetrazolium method on MCF-7 cells (hormone dependent breast cancer cells), DU145 (prostate cancer cells) and H460 (non-small lung cancer cells). Compound **25**, **26**, **27**, **28** and mixture of **29** and **27** exhibited good and potent cytotoxic activity on MCF-7, NCI-H460 and DU145 cell lines. However, **23** and **24** showed no activity toward MCF-7 and NCI-H460 cell lines. **26**, **27**, **28** and mixture of **29** and **27** showed similar pattern of cytotoxic activity toward MCF-7 cell line with IC_{50} values of 3.9 ± 0.8, 3.1 ± 0.1, 5.8 ± 1.2 and 3.0 ± 0.2 μg/mL, respectively. Similar patterns of cytotoxic activity were also observed when **25**, **26**, **27**, **28** and mixture of **29** and **27** tested on NCI-H460 cell lines. The compounds showed IC_{50} values of 13.4 ± 1.1 μg/mL, 5.0 ± 1.2 μg/mL, 1.4 ± 0.9μg/mL, 4.5 ± 1.4 and 2.0 ± 0.7 μg/mL, respectively. **26**, **27**, **28** and mixture of **29** and **27** showed similar pattern of cytotoxic activity toward DU145 cell line with their IC_{50} values of **4.6** ± 0.2, 2.6 ± 0.6, 4.3 ± 0.4 μg/mL and 3.0 ± 0.4 μg/mL, respectively.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGASINGAN BERPANDUKAN BIOCERAKIN DAN PENGENALPASTIAN SEBATIAN-SEBATIAN BIOAKTIF DARIPADA DAUN *GARCINIA PENANGIANA*.

Oleh

MOHD LIP BIN JABIT

Disember 2005

Pengerusi : Professor Nordin Hj Lajis, PhD

Institute : Biosains

Kajian awal yang telah dilakukan ke atas 18 ekstrak pelbagai bahagian *Garcinia sp.* Ekstrak tersebut telah diuji aktiviti sitotoksik menggunakan kaedah mikrotitratan (MTT Tetrazolium) terhadap sel MCF-7 (Sel kanser payudara yang bergantungan dengan hormon), DU145 (Sel kanser prostat), H460 (Sel kanser paru-paru) dan HL-60 (Sel kanser Leukimia). Ekstrak daun *G. penangiana*, daun *G. urophylla*, daun *G. maingayi*, batang *G. maingayi* dan buah *G. opaca* didapati mempunyai aktiviti sitotoksik terhadap MCF-7 dan IC_{50} masing-masing adalah 5 μg/mL, 3 μg/mL, 6 μg/mL, 10 μg/mL dan 8 μg/mL. Seterusnya, ekstrak daun *G. penangiana* juga didapati menunjukkan aktiviti sitotoksik yang tinggi terhadap sel H460 (IC_{50} = 8 μg/mL).

Pengasingan dan penulenan ekstrak daun *G. penangiana* berpandukan biocerakin telah membawa kepada penemuan sebatian triterpena, sterol dan lima sebatian xanthone. Sebatian triterpene dan sterol masing-masing telah dicirikan sebagai friedelin (**23**) dan stigmasterol (**24**). Dua sebatian xanthone yang diasingkan dari fraksi heksana telah

dicirikan sebagai 1,3,5,8-tetrahidroksi-4-(1,1-dimetilallil)xanthone (**25**) dan cudratricusxanthone H (**26**). Dua sebatian xanthone yang diasingkan dari fraksi diklorometana telah dicirikan sebagai 1,3,5,6- tetrahidroksi-2-(1,1-dimetilallil)-4-(3-metil-2-butenil)xanthone atau macluraxanthone C (**27**) dan xanthone baru, penangianaxanthone(**28**). Sebatian **29** juga diasingkan dari fraksi diklorometana dalam bentuk campuran bersama **27**. Biosintesis bagi **25**, **26** and **28** telah dicadangkan di dalam perbincangan. Sebatian-sebatian tersebut diuji aktiviti sitotoksik menggunakan kaedah mikrotitratan (MTT Tetrazolium) terhadap sel MCF-7 (Sel kanser payudara yang bergantungan dengan hormon), DU145 (Sel kanser prostat) dan H460 (Sel kanser paru-paru). **25**, **26**, **27**, **28** dan campuran **29** dan **27** menunjukkan aktiviti baik hingga tinggi ke atas sel MCF-7, NCI-H460 dan DU145. Walau pun begitu, **23** dan **24** tidak memberikan aktiviti ke atas sel MCF-7 dan NCI-H460. **26**, **27**, **28** dan campuran **29** dan **27** menunjukkan profil aktiviti sitotoksik yang sama terhadap sel MCF-7 dengan IC_{50} masing-masing 3.9 ± 0.8, 3.1 ± 0.1, 5.8 ± 1.2 and 3.0 ± 0.2 μg/mL. Profil aktiviti sitotoksik yang sama juga diperhatikan apabila **25**, **26**, **27**, **28** dan campuran **29** dan **27** diuji ke atas sel NCI-H460. Sebatian tersebut menunjukkan IC_{50} masing-masing 13.4 ± 1.1, 5.0 ± 1.2, 1.4 ± 0.9, 4.5 ± 1.4 and 2.0 ± 0.7 μg/mL. **26**, **27**, **28** dan campuran **29** dan **27** menunjukkan profil aktiviti sitotoksik yang sama terhadap sel DU145 dengan IC_{50} masing-masing 4.6 ± 0.2, 2.6 ± 0.6, 4.3 ± 0.4 dan 3.0 ± 0.4 μg/mL.

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I must also thank the staff and students of Natural Products Laboratory, Animal Tissue Culture Laboratories at Institute of Bioscience and Animal Tissue Culture Laboratory at Department of Biomedical Sciences, Faculty of Medical and Health Sciences, University of Putra Malaysia.

Last but not the least, is my utmost, and heart-felt gratitude to my beloved wife and sons, parents and sister for their unremitting love, encouragement, inspiration and continuous support which inspired me to accomplish this work time.

I certify that an Examination Committee has met on 30th December 2005 to conduct the final examination of Mohd Lip bin Jabit on his Master of Science thesis entitled "Bioassay-Guided Isolation and Identification of Bioactive Compounds from *Garcinia penangiana* Leaves" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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(Internal Examiner)

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School of Graduate Studies
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Date: 26 APR 2006

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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(Member)

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Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia.

Date: 11 MAY 2006

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

MOHD LIP BIN JABIT

Date: 25 April 2006

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LIST OF ABBREVIATIONS

µg/mL	Microgram per mililitre
µ L	Microlitre
CGM	Complete growth medium
$CHCl_3$	Chloroform
DMSO	Dimetylsulfoxide
ED_{50}	50% Effective dose
HePG2	Human hepatocellular carcinoma
IC_{50}	50% Inhibitory concentration
LL/2	Mouse Lewis lung carcinoma
mL	Mililitre
MeOH	Methanol
MOLT4	Lympoblastic leukemia
P388	Mouse Leukemia
TLC	Thin Layer Chromatography
VLC	Vacum liquid chromatography
WEHI1640	Mouse fibrosarcoma

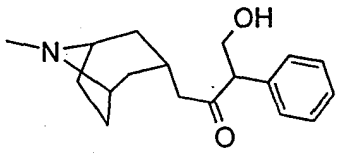
CHAPTER 1

INTRODUCTION

Plants as a Source of Medicinal Agent

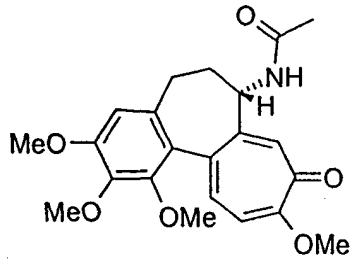
Man has utilized plants as medicinal agent since the history of humankind itself. The oldest record comes from Mesopotamia and dated from about 2600 BC where at least one thousand types of plants have been used in drug formulations (Newman *et al.*, 2000).

It is only in the early 19th century that the active principles from plants were isolated. There are several notable active principles isolated from plants such as atropine (**1**), colchicine (**2**), morphine (**3**) and strychnine (**4**).



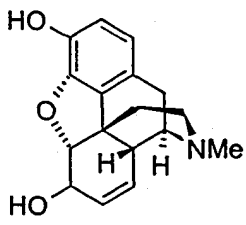
1

Atropine



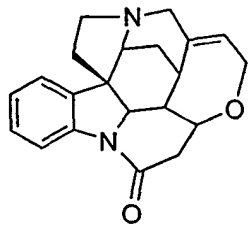
2

Colchicine



3

Morphine



4

Strychnine

A report by the World Health Organization (WHO) claimed that about 80% of the world's population still relies on traditional medicine for treatment of disease or health sustenance (Farnworth, 1985). This is not surprising since such medicinal remedy is cheaper and believed to be safer than the modern medicines. However, there is also the possibility that the herb used in the traditional medicine is harmful and thus treatment may do more harm than good (Elvin-Lewis, 2001). There is also the possibility that the herbs used are not effective at all. Cragg *et al.* (1997) reported that between 1983 and 1994, 41% of new drugs approved by Food and Drug Administration (FDA) have natural products as their sources (Figure 1).

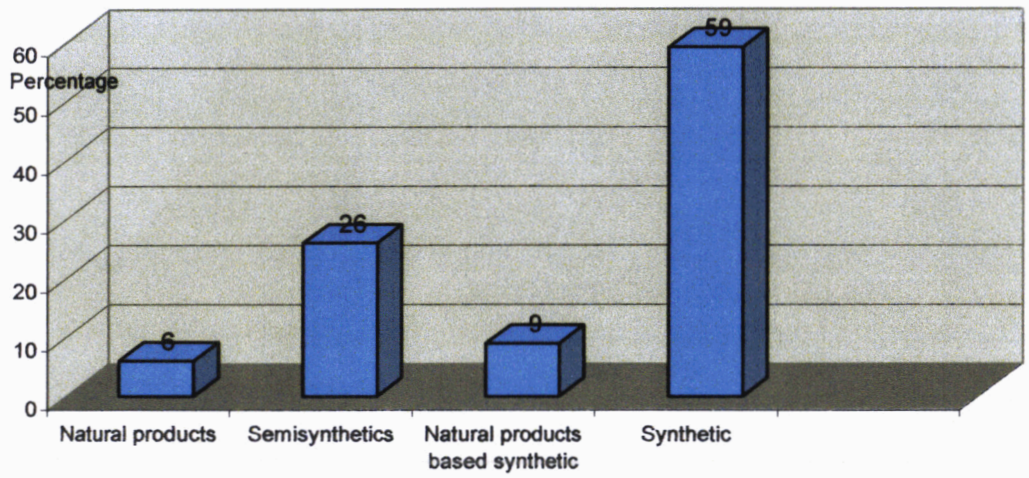


Figure 1: The role of drugs in modern medicine.

These included the semisynthesis and natural products based on synthesic drugs. Scientists continue to investigate the active compounds from plants, which are involved in so many bioactivities, such as antiinflammatory (Nakatani *et al.*, 2002), anti-HIV (Lin *et al.*, 1997), antibacterial (Permana *et al.*, 2001; Rukachaisirikul *et al.*, 2003) and